

investigated by *in vivo* ^{31}P -NMR

Hans-O. Pörtner, Christian Bock and Anke Reipschläger
 Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany

Introduction:

pH dependent modulation of the energy demand of acid-base regulation was studied by *in vivo* ^{31}P -NMR. This study was stimulated by previous findings of a change in metabolic rate with decreases in extracellular pH (pHe) leading to the hypothesis that the cost of acid-base regulation may be modified by a shift between ion exchange mechanisms (1). It was possible to investigate the response of acid-base regulation to hypercapnic conditions and the mechanisms involved. We present *in vivo* measurements in isolated muscle of a marine invertebrate (*Sipunculus nudus*) over days (!) without any degradation of the tissue. To our knowledge this is the first study to report pHe dependent fluctuations in the energy demand of acid-base regulation in vertebrate or invertebrate organs.

Methods:

Large specimens (20-40g) of the marine peanut worm *Sipunculus nudus* were collected from sandy sediments of the intertidal zone in Brittany, France in February 1997 and kept in aquaria as described previously (1). For the preparation of isolated body wall musculature, animals were killed by decapitation behind the base of the introvert retractor muscles. The animals were opened and all inner organs including the ventral nerve cord removed. Muscle tissue slices measuring about 2 times 3cm were fixed onto a plastic grid using surgical thread and placed in a perfusion chamber. 400ml of artificial sea water (34‰ salinity) were thermostatted to 15°C and recirculated through the perfusion chamber and a water reservoir using Tygon tubing and a roller pump. Under control conditions the water reservoir was equilibrated with 40% air and 60% nitrogen supplied by a gas mixing pump (1). Hypercapnia was introduced by switching to a mixture of 50% air, 48% nitrogen and 2% CO_2 and by supplementing the artificial sea water with NaHCO_3 as required for the adjustment of water pH (considered equivalent to pHe). Experiments were performed at pHe 7.9 ($n=4$) and 7.5 ($n=3$). pHe was checked at the beginning and at the end of each experiment and confirmed to remain stable within ± 0.05 units of the initial value. Inside the magnet the muscle tissue was allowed to equilibrate for about 12 hours to the respective pHe before starting hypercapnic incubations. Hypercapnic conditions lasted for up to 72h depending on the changes in the NMR spectra. After reaching steady state values of pH_i normocapnic conditions were reestablished at the same pHe. Subsequently, hypercapnic exposure was repeated under the addition of transport inhibitors, 5-(*N,N*-dimethyl)-amiloride (DMA) for the inhibition of Na^+/H^+ -exchange or 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) for the inhibition of Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ -exchange. Oxygen consumption was studied by use of Eschweiler Clark type electrodes.

^{31}P -NMR studies (e.g. 2) were performed at 81 MHz using a 47/40 Bruker Biospec DBX system with actively

shielded gradient coils. RF pulses were transmitted by using a 5.0cm diameter $^1\text{H}/^{31}\text{P}/^{13}\text{C}$ surface coil placed directly under the perfusion chamber. ^{31}P -NMR spectra were recorded continuously with a sweep width of 4000Hz, 60° bp-pulses of 50 μs and a repetition time of 1.2s for 1000 scans resulting in an acquisition period of 20 minutes.

Results and Discussion:

Within the first 12 hours under control conditions inorganic phosphate (P_i) levels decreased and phospho-L-arginine (PLA) levels increased to steady state levels. Experiments could be continued for 5 days without significant changes in the ^{31}P -spectra under control conditions indicating that tissues remained viable. With the onset of hypercapnia intracellular pH (pH_i) decreased but returned to nearly control values ($\text{pH}_i=7.35$) within about 10 hours and remained constant thereafter (Figure 1a). Returning to control conditions induced a slight overshoot in pH_i before control values were re-established. During hypercapnia at pHe= 7.5 (Figure 1b) pH_i recovered only within 40h. These results indicate a partial inhibition of proton equivalent ion exchange at low extracellular pH. Inhibition of the Na^+/H^+ - exchanger by DMA delayed recovery at pHe= 7.9, but was less effective at pHe=7.5. The addition of the transport inhibitor DIDS prevented pH_i recovery at pHe=7.5 but not at pHe=7.9 when this process was only delayed. These results indicate that the $\text{Na}^+/\text{H}^+/\text{Cl}^-/\text{HCO}_3^-$ exchanger is active at both pHe values but becomes predominant at pHe=7.5. The data are in line with the hypothesis that a shift may occur from the Na^+/H^+ - to $\text{Na}^+/\text{H}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange under conditions of extracellular acidosis (1). ATP stoichiometries of these exchangers suggest a falling ATP demand of acid-base regulation during acidosis, as confirmed by falling rates of oxygen consumption under the effect of falling pHe and of the respective inhibitors.

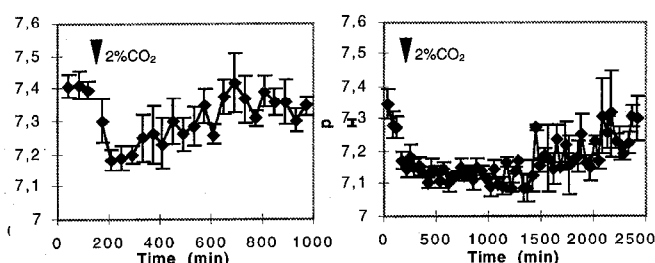


Figure 1: Time course of pH_i changes in muscle tissue under normocapnic and hypercapnic conditions at $\text{pH}_e=7.9$ and $\text{pH}_e=7.5$.

References:

- 1) Reipschläger A, Pörtner HO, J. Exp. Biol. 199: 1801-07 (1996)
- 2) Van den Thillart G, van Waarde A, Physiol Rev. 76: 799-837 (1996)